Organic & Biomolecular Chemistry

This article is part of the

OBC 10th anniversary

themed issue

All articles in this issue will be gathered together online at

www.rsc.org/OBC10

Organic & Biomolecular Chemistry

Cite this: *Org. Biomol. Chem.*, 2012, **10**, 5760

www.rsc.org/obc

COMMUNICATION

New H-bonding patterns in biphenyl-based synthetic lectins; pyrrolediamine bridges enhance glucose-selectivity†

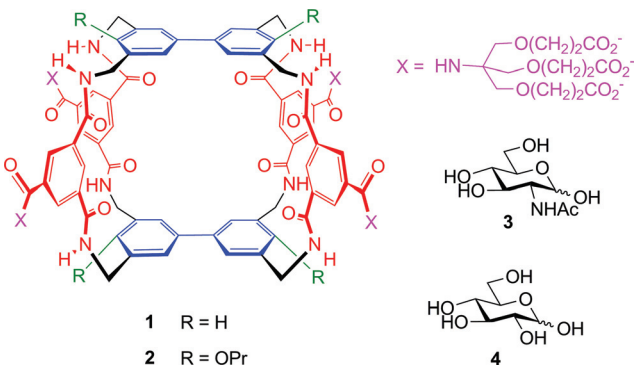
Gururaj Joshi[a,b] **and Anthony P. Davis***[a]

Received 10th May 2012, Accepted 13th June 2012
DOI: 10.1039/c2ob25900a

Synthetic lectins are molecules designed for the challenging task of biomimetic carbohydrate recognition in water. Previous work has explored a family of such systems based on bi/terphenyl units as hydrophobic surfaces and isophthalamide spacers to provide polar binding groups. Here we report a related receptor which employs a new spacer, 2,5-bis-(aminomethyl)-pyrrole, with an alternative (A-D-A) set of H-bonding valencies. The modified spacer leads to significant changes in binding selectivity, including a preference for glucose over all other tested substrates.

The design of biomimetic receptors for carbohydrates in water remains an important challenge.[1] On the one hand, saccharides are problematic targets. Typically they are coated with hydroxyl groups and are therefore highly hydrophilic. They are also "hydromimetic" in their resemblance to clusters of water molecules, and are therefore especially difficult to distinguish from competing solvent. Indeed, the affinities of lectins (carbohydrate-binding proteins) for their substrates are notoriously weak on the general scale of biomolecular interactions.[2] On the other hand, despite the low affinities, natural carbohydrate recognition is highly important.[3] Protein–carbohydrate interactions play roles in crucial biological processes such as fertilization,[3c–g] neuronal development,[3d–h] hormonal activities,[4] tumour metastasis,[5] immune surveillance[6] and inflammatory responses.[7] Synthetic receptors can throw light on the principles which underlie lectin binding, could potentially complement these proteins as research tools for glycobiology, and may eventually find applications in medicine.[1c,8]

Over the past few years we have explored a family of synthetic lectins based on pairs of biphenyl and terphenyl units linked to form cages using isophthalamide spacers.[9] Exemplified by prototype **1** these molecules are designed for complementarity to the β-glucosyl family of carbohydrates, *i.e.* those with all-equatorial substitution patterns. The bi/terphenyl units provide hydrophobic surfaces, complementary to the axial CH groups in the carbohydrates, while the isophthalamides contain H-bond donor/acceptor units positioned to interact with polar groups in the substrates. Given the challenging nature of the problem, these receptors have performed remarkably well. For example prototype **1** binds β glycosides of *N*-acetylglucosamine (GlcNAc) **3** with K_a up to 1000 M^{-1} and selectivities ≥20 : 1 *vs.* other monosaccharides. Meanwhile alkoxy-substituted **2** prefers glucose **4**, binding this important substrate with K_a = 60 M^{-1}, and selectivity ~20 : 1 *vs.* axially-substituted carbohydrates.



To date, our exploration of this system has been restricted to changes in the parallel hydrophobic surfaces. Thus we have extended the surfaces (from biphenyl to terphenyl) to bind disaccharides,[9b,f] and have added substituents to moderate π-electron density and (as in **2**) tune selectivities.[9d,e] Throughout this work the polar interactions, provided by the isophthalamide units, have remained unaltered. The strength, nature and disposition of the hydrogen-bonding units could have major effects on the affinities and selectivities of the receptors, so variation of the spacers is an important objective. We now describe the first example of a biphenyl-based synthetic lectin with alternative polar spacer units, and also the first in which all spacers are not the same. The new design has significantly altered selectivity, being the first to show preference for glucose against all other tested carbohydrates.

In this first attempt at spacer variation, we chose to retain the isophthalamides at one end of the receptor while introducing an alternative unit at the other. The second spacer unit needed to be roughly the same size as the isophthalamides (to maintain the shape of the cage) but complementary in other respects. The

[a]*School of Chemistry, University of Bristol, Cantock's Close, Bristol BS1 1TS, UK. E-mail: Anthony.Davis@bristol.ac.uk; Fax: +44 (0) 117 9298611; Tel: +44 (0) 117 9546334*
[b]*Department of Chemistry and Biochemistry, University of Texas at Austin, Austin, Texas 78712, USA*

†Electronic supplementary information (ESI) available: Experimental details, spectra for compound characterisation and binding studies, and fitting curves for binding analyses. See DOI: 10.1039/c2ob25900a

group of Roelens had previously reported the cage **5** as a powerful and selective receptor for lipophilic β-glucosides in chloroform.[10] As illustrated in Fig. 1, the 2,5-bis-(aminomethyl)-pyrrole (BAMP) spacers in **5** are similar in length to isophthaloyl units but very different in terms of H-bonding characteristics. While the isophthalamides can furnish the binding site with two H-bond donor units (Fig. 1a) or a donor–acceptor combination (Fig. 1b),[11] the BAMP linkers contribute a centrally located donor flanked by two acceptors (Fig. 1c) or (after protonation) a further two donors (Fig. 1d). In water at pH 7 the BAMP unit should be bis-protonated, but the deprotonated form could readily be accessed and studied under basic conditions. Thus BAMP seemed well-suited to serve as a complementary spacer in this research. The spacers would need to support the isophthalamides in maintaining water solubility, so carboxylate groups in the pyrrole 3 and 4 positions were added to the design.

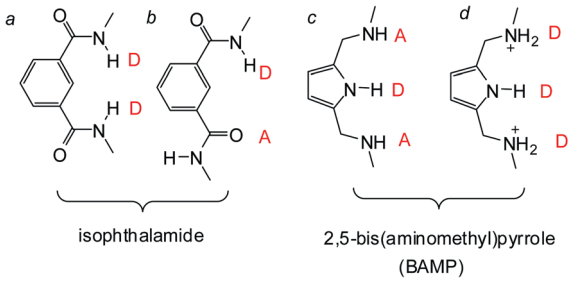
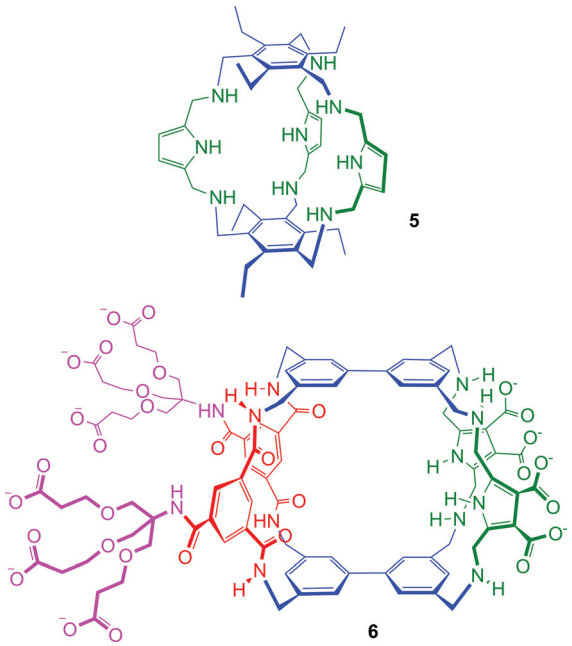
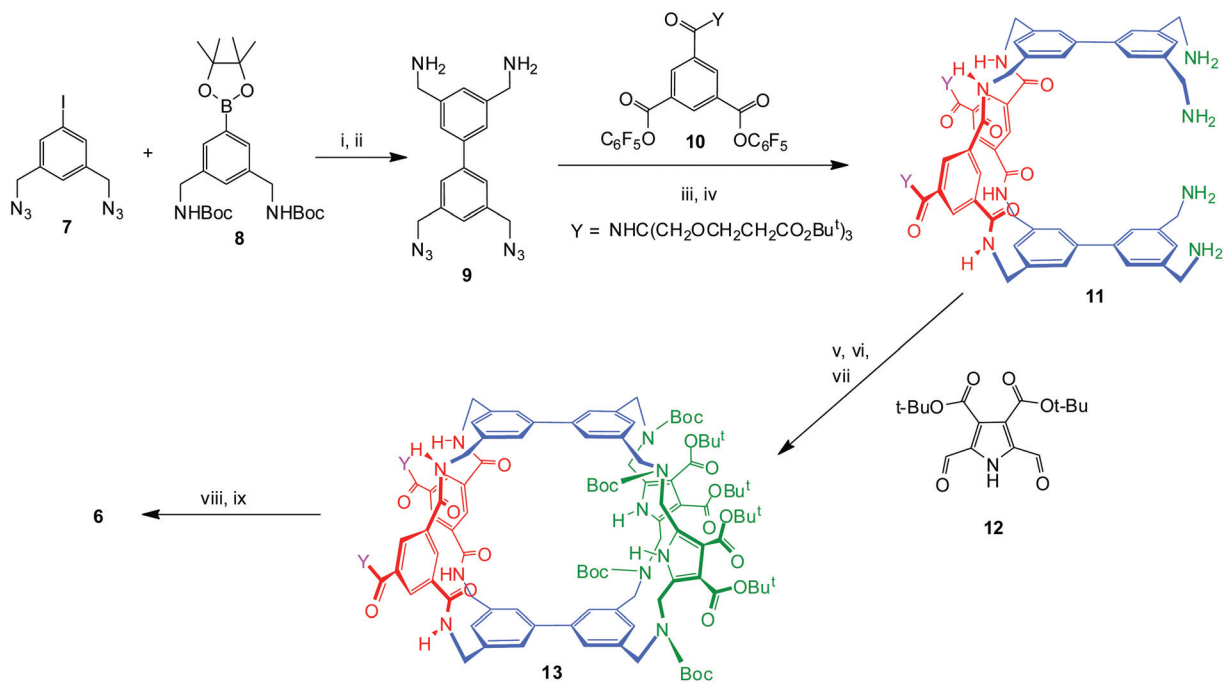


Fig. 1 H-bonding characteristics of the isophthalamide and BAMP spacers.

Macrotricycle **6** thus became the focus of this work.



Receptor **6** was prepared following the route shown in Scheme 1. Suzuki–Miyaura coupling of iodo-bis-azide **7**[9f] with boronate **8**[12] gave an orthogonally protected biphenyl derivative, which was treated with TFA to remove Boc groups. The resulting diamine **9** was allowed to react with bis-pentafluorophenyl ester **10**[9a] under high dilution, to give the corresponding [2 + 2]



Scheme 1 (i) DMSO, $Pd(dppf)_2Cl_2$, NaOAc, 80 °C, 6 h, 77%; (ii) DCM, TFA, 5 h, 97%; (iii) **10**, THF, DIPEA, high dilution (1.2 mM), 30 h, 55%; (iv) THF/MeOH sat. NH_3, Pd/C, H_2 (1 atm), 2 h, 94%; (v) **12**, $CHCl_3$–MeOH 9 : 1, overnight; (vi) MeOH, $NaBH_4$, 2 h; (vii) THF, $(Boc)_2O$, 55 °C, overnight, 34% over three steps; (viii) DCM, TFA, 5 h, 93%; (ix) MeOH/H_2O, NaOH, 99%. DCM = dichloromethane, Boc = *tert*-butoxycarbonyl, DIPEA = diisopropylethylamine, DMSO = dimethylsulfoxide, TFA = trifluoroacetic acid, dppf = 1,1′-bis(diphenylphosphanyl)ferrocene.

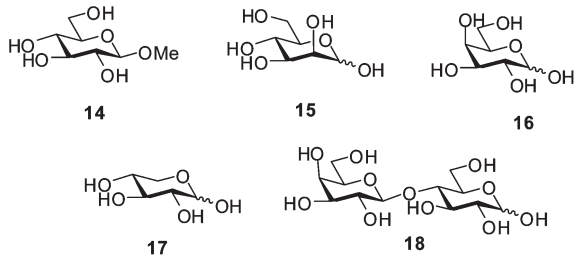


Fig. 2 Carbohydrates tested for their affinities with **6**.

macrolactamisation product. Hydrogenolysis of the four azido groups in this macrocycle (H_2, Pd/C) yielded tetra-amine **11**. Following the lead of Roelens *et al.*,[10] the BAMP spacer units were introduced through reductive amination. Condensation of **11** with dialdehyde **12**[13] in $CHCl_3$–CH_3OH 9 : 1 gave an apparently clean transformation (by ^{1}H NMR) to the corresponding macrotricyclic tetra-imine. Reduction with $NaBH_4$ followed by protection with di-*tert*-butyl dicarbonate (to aid isolation and characterisation) gave macrotricycle **13** in 34% yield over 3 steps. Finally the Boc protecting groups and *t*-butyl esters were cleaved using DCM/TFA, and the resulting decacarboxylic acid was neutralised with NaOH to pH = 7, followed by lyophilisation. Unfortunately the resulting solid, presumably tetra-*N*-protonated **6**, was insoluble in water. Thus the effect of bis-protonated BAMP spacers, as in Fig. 1d, could not be studied. However the receptor was readily soluble in D_2O at pD = 13, giving clean and well-resolved NMR spectra corresponding to **6**. It was therefore possible to test this structure, with native BAMP spacers (Fig. 1c), as a carbohydrate receptor.

The binding of macrotricycle **6** to carbohydrates **3**, **4** and **14–18** (Fig. 2) was studied by ^{1}H NMR titration at pD = 13 in D_2O. Typically the addition of carbohydrate substrates caused movements of most of the receptor aromatic signals, consistent with complex formation in fast-medium exchange on the ^{1}H NMR chemical shift timescale. Fig. 3a shows portions of the NMR spectra resulting from the titration of **6** with D-glucose **4**, in which the movements of protons *B*, *C*, *E* and *F* are especially pronounced (see Fig. 3a for key). The movements were analysed by non-linear curve-fitting according to a 1 : 1 binding model, using an Excel spreadsheet. The fit between observed and calculated data was generally good, as illustrated for **6** + **4** in Fig. 3b, supporting the assumption of 1 : 1 stoichiometry.

The binding constants resulting from these analyses are listed in Table 1, along with the corresponding values for prototype receptor **1**. The first point to note is that **6** is a fairly effective receptor, binding glucose **4** with K_a = 18 M^{-1} (roughly twice as strongly as **1**). The increase is achieved despite a lowering of symmetry from D_{2h} to C_{2v}. This reduces the number of equivalent binding modes from 4 to 2, with a consequent loss of binding entropy.[14] At the same time, the change from isophthalamide to BAMP spacers has significant consequences for selectivity. While the affinity for glucose has increased, binding to GlcNAc **3** has been considerably weakened. Also much lower is the affinity for methyl β-D-glucoside **14** which is bound >4 times less strongly than glucose itself. The change in structure thus seems to favour glucose itself over other all-equatorial substrates. The weak binding to β-glucoside **14** is especially interesting as this has previously been a good substrate for biphenyl-based receptors[9a,c–e] (not unexpectedly, given its all-equatorial configuration and hydrophobic nature). The result may imply a particular mode of binding for glucose which is not available to **14** for steric reasons, or may involve specific polar interactions with the reducing anomeric centre. The geometry of the cavity

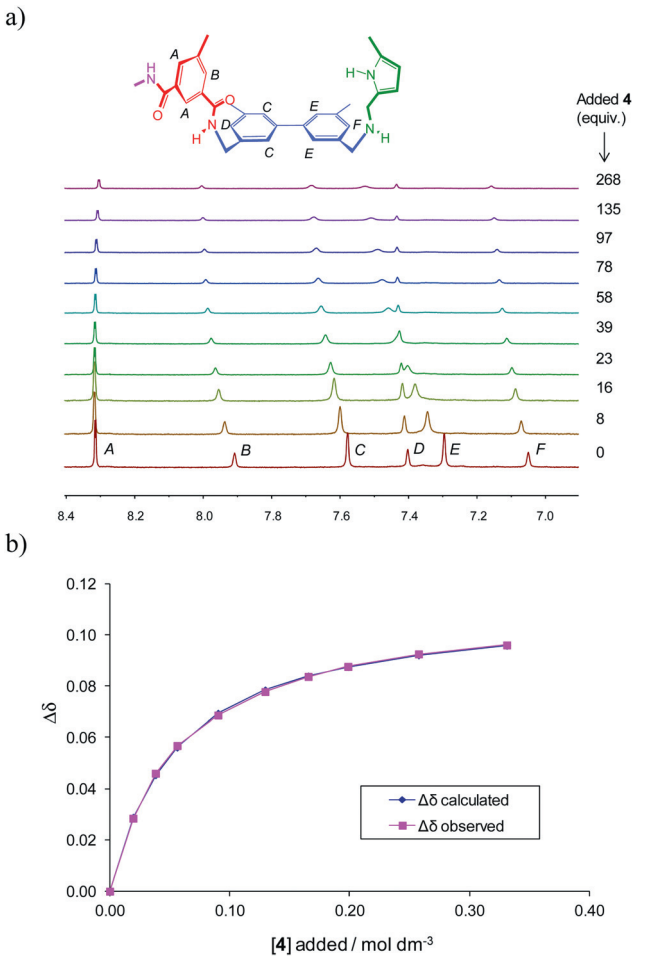


Fig. 3 ^{1}H NMR titration of receptor **6** with D-glucose **4** in D_2O at pD = 13. (a) Partial spectra, with assignments. (b) Observed and calculated binding curves for proton *B*; K_a = 18 M^{-1}.

Table 1 Binding constants K_a for 1 : 1 complexes of receptor **6** with carbohydrate substrates in D_2O as determined by ^{1}H NMR titrations. Data for **1** is given for comparison[a]

	K_a [M^{-1}]	
Carbohydrate	**1**[b]	**6**
D-Glucose **4**	9	18
N-Acetyl-D-glucosamine (GlcNAc) **3**	56	7
Methyl β-D-glucoside **14**	28	4
D-Mannose **15**	~0[c]	~0[c]
D-Galactose **16**	2	3
D-Xylose **17**	5	4
D-Lactose **18**	~0[c]	~0[c]

[a] pD = 13, *T* = 298 K. [b] Values for **1** from ref. 9c. [c] Too small for reliable analysis.

may play a role, although molecular modelling[15] on **6** predicted an open conformation with similar dimensions to that of **1**. Whatever the detailed explanation, the change in selectivity is presumably due to the recasting of H-bonding valencies at one end of the cavity, from the D–A or D–D of isophthalamide to the A–D–A of BAMP (Fig. 1a–c).

In conclusion, we report a new type of biphenyl-based synthetic lectin in which, for the first time, the standard isophthalamide spacers have been replaced by units with alternative arrangements of polar binding groups. The recognition properties of this system confirm that such changes are permissible, and can be used to tune selectivities. In this case the result is unprecedented selectivity for glucose against all other tested carbohydrates. The introduction of the BAMP spacer is clean and high-yielding, encouraging its adoption in future designs. In particular it will be interesting to prepare analogues with improved solubility at pH 7, to test the D–D–D H-bonding arrangement in Fig. 1d.

Acknowledgements

This work was supported by the EPSRC (EP/D060192/1) and the Commonwealth Commission (Postgraduate Scholarship to G.J.).

Notes and references

1 For reviews, see: (*a*) A. P. Davis and R. S. Wareham, *Angew. Chem., Int. Ed.*, 1999, **38**, 2978–2996; (*b*) A. P. Davis, *Org. Biomol. Chem.*, 2009, **7**, 3629–3638; (*c*) S. Jin, Y. F. Cheng, S. Reid, M. Y. Li and B. H. Wang, *Med. Res. Rev.*, 2010, **30**, 171–257; (*d*) M. Mazik, *Chem. Soc. Rev.*, 2009, **38**, 935–956. For recent examples, see: (*e*) G. Fukuhara and Y. Inoue, *J. Am. Chem. Soc.*, 2011, **133**, 768–770; (*f*) A. Pal, M. Berube and D. G. Hall, *Angew. Chem., Int. Ed.*, 2010, **49**, 1492–1495; (*g*) M. Rauschenberg, S. Bomke, U. Karst and B. J. Ravoo, *Angew. Chem., Int. Ed.*, 2010, **49**, 7340–7435; (*h*) S. Striegler and M. G. Gichinga, *Chem. Commun.*, 2008, 5930–5932; (*i*) T. Reenberg, N. Nyberg, J. O. Duus, J. L. J. van Dongen and M. Meldal, *Eur. J. Org. Chem.*, 2007, 5003–5009; (*j*) H. Goto, Y. Furusho and E. Yashima, *J. Am. Chem. Soc.*, 2007, **129**, 9168–9174; (*k*) M. Mazik and H. Cavga, *J. Org. Chem.*, 2006, **71**, 2957–2963.

2 E. J. Toone, *Curr. Opin. Struct. Biol.*, 1994, **4**, 719–728; M. Ambrosi, N. R. Cameron and B. G. Davis, *Org. Biomol. Chem.*, 2005, **3**, 1593–1608.

3 (*a*) *The Sugar Code – Fundamentals of Glycoscience*, ed. H.-J. Gabius, Wiley-Blackwell, Weinheim, 2009; (*b*) B. Wang and G.-J. Boons, *Carbohydrate Recognition: Biological Problems, Methods and Applications*, Wiley, Hoboken, 2011; (*c*) A. B. Diekman, *Cell. Mol. Life Sci.*, 2003, **60**, 298–308; (*d*) D. J. Miller, M. B. Macek and B. D. Shur, *Nature*, 1992, **357**, 589–593; (*e*) W. J. Snell and J. M. White, *Cell*, 1996, **85**, 629–637; (*f*) R. Shalgi and T. Raz, *Histol. Histopathol.*, 1997, **12**, 813–822; (*g*) E. Rubinstein, A. Ziyyat, J. P. Wolf, F. Le Naour and C. Boucheix, *Semin. Cell Dev. Biol.*, 2006, **17**, 254–263; (*h*) H. E. Murrey and L. C. Hsieh-Wilson, *Chem. Rev.*, 2008, **108**, 1708–1731.

4 G. Caltabiano, M. Campillo, A. De Leener, G. Smits, G. Vassart, S. Costagliola and L. Pardo, *Cell. Mol. Life Sci.*, 2008, **65**, 2484–2492.

5 K. S. Lau and J. W. Dennis, *Glycobiology*, 2008, **18**, 750–760.

6 E. I. Buzas, B. Gyorgy, M. Pasztoi, I. Jelinek, A. Falus and H. J. Gabius, *Autoimmunity*, 2006, **39**, 691–704.

7 (*a*) R. A. Dwek, *Chem. Rev.*, 1996, **96**, 683–720; (*b*) P. M. Rudd, T. Elliott, P. Cresswell, I. A. Wilson and R. A. Dwek, *Science*, 2001, **291**, 2370–2376; (*c*) D. A. Calarese, C. N. Scanlan, M. B. Zwick, S. Deechongkit, Y. Mimura, R. Kunert, P. Zhu, M. R. Wormald, R. L. Stanfield, K. H. Roux, J. W. Kelly, P. M. Rudd, R. A. Dwek, H. Katinger, D. R. Burton and I. A. Wilson, *Science*, 2003, **300**, 2065–2071.

8 J. Balzarini, *Nat. Rev. Microbiol.*, 2007, **5**, 583–597.

9 (*a*) E. Klein, M. P. Crump and A. P. Davis, *Angew. Chem., Int. Ed.*, 2005, **44**, 298–302; (*b*) Y. Ferrand, M. P. Crump and A. P. Davis, *Science*, 2007, **318**, 619–622; (*c*) Y. Ferrand, E. Klein, N. P. Barwell, M. P. Crump, J. Jiménez-Barbero, C. Vicent, G.-J. Boons, S. Ingale and A. P. Davis, *Angew. Chem., Int. Ed.*, 2009, **49**, 1775–1779; (*d*) N. P. Barwell, M. P. Crump and A. P. Davis, *Angew. Chem., Int. Ed.*, 2009, **48**, 7673–7676; (*e*) N. P. Barwell and A. P. Davis, *J. Org. Chem.*, 2011, **76**, 6548–6557; (*f*) B. Sookcharoenpinyo, E. Klein, Y. Ferrand, D. B. Walker, P. R. Brotherhood, C. Ke, M. P. Crump and A. P. Davis, *Angew. Chem., Int. Ed.*, 2012, **51**, 4586–4590.

10 O. Francesconi, A. Ienco, G. Moneti, C. Nativi and S. Roelens, *Angew. Chem., Int. Ed.*, 2006, **45**, 6693–6696.

11 A third conformation with two inward-directed carbonyl oxygens is possible in principle. However this is less likely as it moves the biphenyl surfaces too far apart for effective interaction with a carbohydrate (see also ref. 9*c*).

12 T. J. Ryan, G. Lecollinet, T. Velasco and A. P. Davis, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 4863–4866.

13 M. Galezowski and D. T. Gryko, *J. Org. Chem.*, 2006, **71**, 5942–5950.

14 L. Challinor, E. Klein and A. P. Davis, *Synlett*, 2008, 2137–2141.

15 Monte Carlo molecular mechanics conformational search, employing MacroModel 9.9 (MMFFs force field, water GB/SA solvation) accessed *via* the Maestro 9.2 interface.